Chapter 2

Bacteria in Soil

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I. INTRODUCTION

Bacteria are the smallest and the most numerous of the free-living micro-organisms in soil. Taken collectively, their spectrum of autotrophic and hetero-trophic capabilities is matched by none other of the major groups of the soil life. It belies their small size and comparatively simple morphology. Notwith-standing the abundance of bacteria in soil and extensive information about the activities of individual species in standardized environments, the role of bacteria in many soil processes is still poorly understood. Much also remains unknown concerning the forms of bacteria in soil and their ecological relation-ships within their micro-environments.

Commonly the soil bacteria are discussed according to their participations in the nitrogen and carbon cycles or in other cyclical transformations in soil. Discussions of them may also be undertaken on the basis of their morphology and taxonomy, their environmental tolerances, their spatial distribution in soil, and from the standpoint of their biotic relationships with other micro-organisms or with higher plants. Several of these approaches are used in

subsequent chapters. It is the purpose of this chapter to consider the bacteria *en masse* in the soil, with little or no effort to spell out the many special problems posed by individual genera or species.

II. THE NUMBER OF BACTERIA IN SOIL

The number of bacteria in 1 g of soil ranges from 1 million or less to several billions.* That their population needs to be expressed within such extremes is in part due to the great differences in populations that do exist among and within soils. Somewhat more disconcerting is the fact that with existing methodology, the bacterial population cannot be determined with precision in any given soil sample. Estimation, not measurement, is accomplished.

Estimates are made culturally or by direct microscopic examination. The cultural method most commonly employed is the dilution plate technique. A primary assumption in this technique is that each viable bacterium in the soil suspension used as an inoculant develops into a visible colony during incubation. Any such assumption is highly idealistic. It is not possible to prepare any one combination of substrate ingredients and conditions of incubation that will permit the growth of all bacterial species. Even with the cultural conditions made as optimal as knowledge permits for a given species, not all viable cells of that species will invariably produce colonies. Due to differences in stored reserves and enzymatic activity, or in dormancy or senescence, the individual cells are not equally vigorous in initiating growth. There also are differences in their micro-environments within the cultural substrate, as for example, proximity or lack of proximity to other microbial cells.

That collectively these several factors can suppress colony formation by the majority of bacterial cells seeded into agar has been shown by Tchan (1952). A known number of cells was taken from a young, actively growing culture of Azotobacter and transplanted into glucose agar. As microscopic examination of the inoculant showed all cells to be actively motile, all the seeded cells were considered to be viable. The number of colonies subsequently developing represented only 25–33% of the number of bacterial cells known to have been seeded. If only one cell in three or four of a vigorously growing bacterium such as Azotobacter can successfully accomplish colony formation in a substrate designed especially for this organism, then it would indeed be surprising if more than one cell in ten, or even one in a hundred, of the wide assortment of bacteria in soil could initiate colony formation when seeded jointly on any single substrate under any given set of incubation conditions.

Soil bacterial populations as determined by cultural methods range from a few thousand cells per gram of soil to several hundred millions. Sand dunes, tundras, stony soils, and podsols usually contain comparatively low, and chernozems and other prairie soils, comparatively high bacterial populations. Commonly for arable soils cultural methods of examination reveal from

* One billion as used by the author equals 10^9.

5–50 million bacteria/g of soil. In rare instances bacterial populations as high as 500 million/g have been determined for field soil by cultural methods (Krasilnikov, 1958).

Even this value, however, hardly approaches the population estimates made by direct microscopical procedures. Strugger (1948), using fluorescent microscopy, observed from 2–9 billion viable cells/g. Taylor (1936), working with Rothamsted soils and using the dilution ratio method for direct counting, observed soil bacterial populations of approximately 3 billion/g. Direct counts were of the order of 10–50 times the magnitude of the agar plate counts made on the same samples. Jensen (1936) reported quite similar results for Australian soils. Direct counts were from 8–300 times higher than plate counts. The widest ratios between direct and cultural counts were found in soils receiving no recent additions of energy-rich material, such as green manure. Immediately following addition of an organic material to soil, the ratio between the two counts was narrowed very markedly. It frequently became less than 10 and at times approached unity.

Citations such as the preceding indicate that the soil bacterial population as determined by direct microscopy is usually of the order of 1–3 billion/g. In highly fertile soils and for short periods, the value may approach 10 billion. For purposes of discussion in some immediately following paragraphs, 2 billion cells/g will be taken as a representative value for the bacterial population of soil. Also, a representative bacterial cell in soil will be considered as spherical and to have a cell volume of 1 μ^3. These two generalizations permit making some estimates of the weight of bacteria in soil and of the extent to which bacteria occupy the available space in soil. The standard population estimate will also be used for considering the suitability of soil as a *milieu* for bacterial growth.

III. THE BACTERIAL BIOMASS AND ITS SPATIAL REQUIREMENT

On the basis of either a cell volume of 1 μ^3 and a cell density only slightly in excess of unity (1·04 according to Kendall, 1928) or on the common generalization that 1 trillion bacterial cells weigh 1 g (Pelczar and Reid, 1958), a direct count estimate of 2 billion bacterial cells/g of soil accounts for 0·2% of the soil weight. This amounts to 4,000 lb live weight of bacteria/acre 6 in. For many arable soils, the assumed bacterial population value, and therefore the live weight value per acre are probably over-estimates, but not markedly so. Most writers prefer to state a general range of values when speaking of the amount of bacterial tissue per acre. Alexander (1961) has stated the range as from 300–3,000 lb/acre-furrow slice; Russell (1950), as from 1,500–3,500 lb; and Krasilnikov (1944), as 600–1,200 lb for uncropped soils, to as high as 3,600–6,400 lb for soil cropped to legumes.

In most arable soils, the amount of living bacterial tissue per acre is commonly estimated to be somewhat less than that of the fungi, but to exceed that of the algae, protozoa, and nematodes combined. The matter of

comparative weight, or biomass, in relation to population numbers merits emphasis. Although on a census basis the *Eubacteriales* may outnumber the *Actinomycetales* by a factor of 10, and the fungi by a factor of 100, the eubacterial biomass in many cultivated soils is about equal to that of the actinomycetes, and only about half that of the soil fungi.

To what extent do bacteria occupy the available space in soil? First, this can be calculated using the assumption that normally the bacteria exist in the water film in soil. For a soil containing 0·3 ml water/g, a bacterial population of 2 billion will occupy no more than 1·33% of the theoretically available space. This calculation is based on the fact that spheres, regardless of size other than the provision that they be of uniform size, when uniformly open or cubic-packed within a container, occupy approximately one-half the container volume. Accordingly, in 0·3 ml presumably there could occur 150 billion cells. If, however, the bacterial cells are sufficiently plastic that they pack solidly, then the estimated 1·33% occupancy figure becomes halved. Obviously also, if there were nothing to inhibit growth, the bacteria would continue to multiply until they packed the total porosity in soil. In such instance, there could occur something like 500 billion bacteria/g of soil, and the standard population postulated would occupy no more than 0·4% of the available space. Even this figure appears discordant with the statement a few lines above that 2 billion bacteria/g constitute only 0·2% of the soil weight. That the two values are not inharmonious becomes apparent when one considers that the space occupied by the mineral particles is not included when calculating the soil space available for occupancy by bacteria.

At first glance, an approximate 0·4 to 1·0% occupancy of available space suggests that the soil is not an especially suitable environment for bacteria. Accordingly, it becomes fitting to compare the bacterial density in soil to the populations that are encountered in various other environments. Rahn (1945) has recorded some population densities for soured milk and for fresh human feces, as well as for nutrient broth in which bacterial growth has been allowed to continue to a maximum population level. For all three environments, his estimates are of the order of 1 billion cells/g. This value is entirely comparable to the bacterial population in soil. Rahn's estimates are quite conservative, inasmuch as the writer has made estimates as high as 20 billion/g of decaying plant material as found in the field, and as high as 40 billion/g for fresh feces. Even higher values are on record, for example, 46 billion/g in decomposing green manure (Smith and Humfeld, 1930), and 100 billion/g in an infant's feces (Smith, 1961). Nevertheless, it still must be emphasized that on the basis of its contained population, the soil does appear to be an excellent environment for bacteria.

IV. RESPIRATORY ACTIVITY OF SOIL BACTERIA

Numbers alone, however, are no measure of microbial activity. It is possible that a high population present in an environment, particularly if the population estimate is based on direct microscopy, can be largely dormant or

resting. Some assessment of the general activity level of the soil microbial population can be obtained by considering respiratory activity. This can be done conveniently in terms of carbon dioxide production.

According to Mooney and Winslow (1935), carbon dioxide production in glucose-peptone broth by *Salmonella pullorum* occurs at a rate of 1·14 mg/billion cells per hour during the early logarithmic growth phase. At stationary maximum population, the rate was determined as 0·08 mg. Similar data were obtained by Walker and Winslow (1932) working with *Escherichia coli*. They reported 0·41 to 1·85 mg CO_2/billion cells per hour in the late logarithmic phase, and less than 0·02 mg at the close of the logarithmic phase. Actively multiplying bacterial cells therefore appear capable of producing many times their dry matter weight of CO_2 during 24 hours, whereas in stationary populations the production of CO_2 during 24 hours is approximately the equivalent of dry cell weight.

This latter estimate is in reasonable agreement with some observations on CO_2 production by micro-organisms in soil. Jensen (1936) noted production of up to 0·22 mg CO_2/billion bacteria in 24 hours, or roughly the equivalent of the bacterial dry weight. In general, however, the CO_2 production per billion bacteria during 24 hours amounted to about 0·1 mg. Miller and Schmidt (1961) measured 0·12 mg CO_2/billion bacteria per day in laboratory soil culture, and Goring and Clark (1952), 0·11 mg, from glucose-amended laboratory sand cultures.

As a first approximation, therefore, it appears plausible to accept Jensen's estimate of 0·1 mg CO_2/billion bacteria per day. This is equivalent to 0·004 mg on an hourly basis, a rate that is appreciably below those cited above for the stationary phase of *S. pullorum* and *E. coli*.

Rate of carbon dioxide production by field soil has been given by Alexander (1961) as 20 lb/acre per day; by Vine, Thompson and Hardy (1942) as 3 l/m^2 per day; and by Krasilnikov (1958) as 2 kg/hectare per hour. These values, if all reduced to a lb/acre per hour basis, become 0·83, 2·2, and 1·78, respectively. Their mean value is 1·6 lb CO_2/acre per hour.

On the basis of two separate premises stated above, namely, that there are 2 billion bacteria/g of soil, and that in soil, bacteria produce CO_2 at the rate of 0·004 mg/hour per billion, then the $1{\cdot}8 \times 10^{15}$ bacteria in an acre-furrow slice should produce 7,200 g CO_2 in 1 hour. This quantity, 15·9 lb, appears entirely too high for any ready reconciliation with the 1·6 lb that has been revealed by some actual field measurements. How best can this discrepancy be resolved?

It appears reasonable and even obligatory to accept the field measurement of 1·6 lb/acre per hour for CO_2 production from soil. Insofar as production by the soil bacteria is concerned, the 1·6 lb must be several times too high, simply because the bacteria constitute only about one-fifth of the biomass in soil and therefore should be held responsible for only part of the total respiratory activity in soil. Accordingly, it appears necessary to challenge the proposition that there are 2 billion cells/g of soil that are producing CO_2 at the rate of 0·004 mg/billion bacteria per hour. Although some cells may be exhibiting

this or some higher level of respiratory activity, the majority of them must be greatly enfeebled or resting cells, or spores, that are exhibiting an exceedingly low level of respiratory activity. Many of these probably would not be determined in the course of making plate count determinations of the soil population.

Acceptance of the possibility that appreciably less than 2 billion bacteria/g of soil are active helps to explain the wide disparity between plate count and direct count estimates of the bacterial population in soil. If, as documented above, the direct count exceeds the plate count by a factor of 50- to 300-fold, with the ratio below 10-fold only in freshly amended soils or in laboratory cultures of bacteria, then perhaps the plate count when used on soil does enumerate the viable and active bacteria somewhat more successfully than much of the raw data would indicate. The plate count appears more compatible with observed rate of CO_2 production by field soil than does the direct count. Bacterial plate counts of 50 million/g are commonly reported to occur in field soils. If this population respires at a rate of 0·004 mg CO_2/billion cells per hour, then in an acre-furrow slice it would produce CO_2 at the rate of 0·4 lb/hour. If one is willing to assume that the soil bacteria produce approximately one-fourth of the 1·6 lb/acre per hour produced by the total microbial population in soil, then this calculation of 0·4 lb/hour appears entirely reasonable.

Irrespective of any such subsidiary calculation, the discrepancy between the 15·9 lb calculated production and the measured 1·6 lb production makes it appear plausible that a major proportion of the possibly 2 billion bacteria in a gram of soil are in a resting or dormant condition, with their respiratory activity at a level appreciably below that of bacterial cells in broth cultures in the stationary phase of their growth cycle. In brief, population and respiratory data considered jointly indicate that soil permits the survival of a great many bacterial cells during periods in which they are not very active metabolically. It is probable that the resting vegetative cells of at least some soil organisms can attain levels of respiratory activity of the order of 0·0004 mg CO_2/billion cells per hour. At this level of activity, bacterial cells in the soil, even if present in such numbers as are suggested by direct microscopical counts, would make only a very minor contribution to the respiratory output field soil of 1·6 lb CO_2/acre per hour.

V. FACTORS LIMITING SOIL BACTERIAL ACTIVITY

If commonly in the soil, many of the bacteria are either inactive or else are showing only a very low level of activity, what are the factors that limit their activity? There is no quick and easy answer to this question. To do it justice, one becomes almost endlessly involved in innumerable interactions concerning many different soil factors variously affecting different bacteria. Any such approach conflicts with the initially announced objective of this discussion and will not be undertaken. Attention in the following paragraphs will be directed in quite general terms to limitations on bacterial activity imposed by

the available food supply and by certain physical and biological factors in the environment. The physical factors given brief attention are those of moisture, aeration, reaction, and temperature.

The principal factor limiting bacterial growth in soil is scarcity of food, or lack of a suitable and available source of energy. Consequently, any addition of fresh energy material to soil almost invariably elicits an increase in bacterial activity. Many, if not most, bacteriologists believe that in the *in vitro* culture of single species of bacteria, the total yield of cells is proportional, over a wide range, to the amount of food available. The situation in soil in the presence of a mixed bacterial flora is probably analogous. Food supply is of paramount importance. The nature of an added energy material influences both the immediacy and the duration of the rise in activity, as well as the specificity of the responding flora.

Nutritionally, the great majority of the soil bacteria are heterotrophic, that is, they use organic compounds synthesized by autotrophic micro-organisms and higher plants both for their energy requirements and as the principal source of their cell carbon. In contrast, the autotrophic bacteria use carbon dioxide as a source of cell carbon and secure their energy by means of inorganic oxidations. Insofar as the nutrition of the heterotrophic bacteria is concerned, the amount of organic matter added to soil by autotrophic bacteria is negligible in comparison to that formed by the photosynthetic activity of higher plants. Although a tremendous tonnage of organic matter is produced yearly, the voracity of the heterotrophic organisms is such that in most soils the annual rate of decomposition balances out quite nicely with the annual rate of production of organic matter. Maintenance of this balance does not require that the soil organisms work at capacity throughout the year. Insofar as the soil bacteria are concerned, the supply of food materials in soil can be said to be perennially inadequate.

In instances where the supply of energy-yielding substrate is in itself adequate, it is possible that a short supply of one or more of the essential mineral nutrients or of necessary growth factors can be limiting for bacterial activity. A great deal of attention has been given to the requirements of bacteria for nitrogen, phosphorus, and other minerals in the course of decomposition of carbohydrates and of organic residues characterized by wide carbon/nitrogen ratios. With such materials, especially when used experimentally under favorable environmental conditions in the field or laboratory, dramatic stimulation of microbial activity can at times be achieved by using supplemental minerals. Data recently published by Stotzky and Norman (1961a, b) provide a good example. The respiratory output of carbon dioxide from a sandy soil treated with glucose only was doubled when nitrogen and phosphorus were used supplementarily. This rate in turn was quadrupled upon addition of sulfur.

A large number of essentially similar experiments could be cited. However, for most soils and with naturally occurring residues, similarly dramatic responses to supplemental minerals should not be expected to occur. In the work of Stotzky and Norman, the glucose amendment was free of sulfur,

and the sandy soil to which the glucose was added in liberal quantity was itself relatively low in sulfur content. Most organic residues reaching field soils contain nitrogen and other minerals in sufficient quantity that supplies of these minerals should be non-limiting or but slightly limiting to bacterial activity. Nevertheless, with more extended study it may be found that limiting supplies of mineral nutrients or vitamins more often restrict decomposition processes in soil than is generally recognized.

Nitrogen is the mineral nutrient most in demand by bacteria in decomposition of carbonaceous residues. Most crop residues containing 1·5% or more of nitrogen need no additional nitrogen to meet the needs of the bacteria. For residues containing a lower level of nitrogen, the bacteria involved in the decomposition need extra nitrogen, particularly during the early stages of decomposition. Such extra nitrogen as is needed is usually available in the soil. Most arable soils in the course of a season produce from about 20 to about 100 lb of available nitrogen. The lower figure would be ample for the nitrogen demand during decomposition of 1 ton, and the latter figure for 5 tons, of straw or stover containing 0·5% nitrogen.

The quantity of residues returned annually to a soil is usually closely related to the nitrogen fertility of that soil. The residues from a poor soil are small and those from a fertile soil are large, and therefore less demand for soil nitrogen occurs in soils which are poor in nitrogen. It is difficult to find field conditions where the nitrogen content of the residues, when coupled with the available soil nitrogen, is not adequate to meet the nitrogen demands of the soil organisms carrying on the decomposition. Accordingly, it is but seldom that addition of nitrogen will accelerate residue decomposition in the field.

The fact that nitrogen is seldom limiting to the bacteria engaged in decomposition of residues does not mean that extreme nitrogen deficiency cannot occur in crop plants growing in soil well endowed with residues of wide carbon/nitrogen ratios. In the presence of the abundant energy material, the soil organisms may demand all the available soil nitrogen, and leave none for the growing crop. Nitrogen fertilization therefore is essential for the crop but not for the bacteria.

The addition of nitrogen to crop residues does not, as is sometimes assumed, result in the retention of any greater percentage of plant residues as soil organic matter. Nitrogen additions to soils over a number of years do often result in a higher level of soil organic matter than would otherwise exist, but this is due to the production of larger crops and more residues, and not to any higher rate of residue conversion to soil organic matter.

VI. DISTRIBUTIONAL PATTERNS OF BACTERIA IN SOILS

Bacteria are not uniformly distributed throughout the soil profile, nor even throughout a single soil horizon. With but few exceptions, their distribution in soil echoes the distribution of soil organic matter. In the soil profile, organic matter content is usually highest in the A horizon and of lesser quantity in

the B and C horizons. A similar profile distribution of bacteria is commonly noted. Data compiled by Starc (1942) are cited in Table I. Essentially similar data have been obtained by various other workers in diverse localities.

There are, of course, many instances in which the standard profile pattern is subject to derangement. One such example is shown in Table II, wherein

TABLE I

Bacterial distribution according to depth in the soil profile (Starc, 1942)

Horizon	Depth (cm)	Aerobic bacteria (millions/g)	Anaerobic bacteria (millions/g)
A_1	3– 8	7·8	1·95
A_2	20– 25	1·8	0·38
A_2–B_1	35– 40	0·47	0·10
B_1	65– 75	0·01	0·001
B_2	135–145	0·001	0·001

TABLE II

Organic matter and bacterial distribution according to depth in a chernozem soil profile (Timonin, 1935)

Horizon	Depth (cm)	Organic matter (%)	Aerobic bacteria (millions/g)	Anaerobic bacteria (millions/g)
A_1	0– 6	8·04	49·2	1·0
A_2	6–12	3·18	131·8	1·0
B_1	12–28	2·41	158·3	10·0
B_2	28–48	1·76	45·3	1·0
C	48–80	0·80	6·0	0·001

the bacterial maximum does not occur at the same profile depth as does the maximum value for organic matter. In some instances, such non-conforming maxima can be due to droughty conditions at the soil surface. In others, the surface horizon may simply be too acid to permit profuse bacterial development. Failure of bacteria to develop in an A horizon containing abundant energy material does not mean that other units of the soil biota, such as fungi or insects, must also fail to flourish.

In soils in which plants are growing, the organic exudates and sloughings emanating from the root surfaces provide an abundant source of energy material, and there results a profuse development of micro-organisms on or near the root surface. This phenomenon is known as the rhizosphere response. Its study has intrigued many workers. The microbiology of the rhizosphere is

discussed by Parkinson in a later chapter. The rhizosphere is mentioned here only to emphasize that plant roots have a profound influence on the occurrence and localization of soil bacteria. Data illustrative of the extent to which bacterial populations are increased in the immediate vicinity of plant roots are presented in Tables III and IV.

TABLE III

Influence of proximity to corn roots on abundance of bacteria (Starkey, 1931)

Description of sample	Total bacteria	Types growing on mannite agar (millions/g)	Radiobacter types
Soil, 15 cm distant	14·2	7·6	0·02
Soil, 5–10 cm distant	25·4	14·0	0·18
Soil close to roots	122·0	91·4	3·22
Root surface material	1,315·6	523·2	14·12

TABLE IV

Influence of proximity to cotton roots on abundance of bacteria (Clark, 1940)

	Hundred thousands/g of		
Description of sample	Total bacteria	Dye-tolerant bacteria	Spores of Bacillus
Soil, 10–15 cm distant	52·6	7·4	17·0
Soil, 5–10 cm distant	47·9	9·0	18·0
Soil, 2·5–5 cm distant	45·6	7·7	17·0
Soil, 0·5–2·5 cm distant	54·7	15·0	10·0
Soil, 0–0·5 cm distant	129·9	91·3	13·7
Root surface scrapings	510·0	440·0	0·1

Additionally to local concentrations of bacteria in the rhizosphere, there can occur islands or foci of microbial activity in soil apart from plant roots. Such foci vary greatly in magnitude. At one extreme is the condition that prevails when a green manure crop is plowed down. In such instance a food supply measurable in tons per acre is layered into the soil. Such layers can contain many billions of bacteria per gram of plant material, while soil but a few centimeters distant, if free of contact with the added plant parts, shows only a few millions per gram (Smith and Humfeld, 1930). On a smaller scale, foci of bacterial activity develop when individual plants or portions of them fall onto or become incorporated into the soil. Following harvest of seed cotton from a field, the writer has observed that decaying cotton carpels in

natural microclimates at the soil surface can support bacterial populations of 20 billion/g plant material.

Other foci of bacterial development are known to be associated with the fecal droppings of insects and with dead mesofaunal and microbial tissues. Microscopic examination of glass slides that have been buried in soil frequently reveals small threads or cylindrical masses of bacterial cells clustered about a disintegrating strand of fungus mycelium. Beyond this level of observation, there exists a relatively little explored area concerning the microdistribution of bacteria in soil. Alexander and Jackson (1954) observed that soil bacteria commonly occur in the film of colloidal material coating the mineral particles in soil. Jackson *et al.* (1947) have published electron micrographs showing small masses of bacteria closely surrounded by clay particles. Corroborative evidence that bacteria occur in small colonies in soil has been noted by Jones and Mollinson (1948). They observed that 77% of the bacteria in soil occurred in groups of several to many cells, while the remaining 23% occurred as single cells. Minderman (1956), on the other hand, examined soil sections and concluded that the majority of the bacterial cells observed therein occurred as single cells.

Some occurrence of solitary bacteria in soil is to be expected. Individual cells must be subject to some transport within the soil by the migratory activities of the soil micro- and mesofauna. A certain amount of self-tillage occurs in soil coincidentally to gross changes in soil moisture and temperature. Movement of gravitational water provides another possibility for bacterial transport in soil. According to Thornton and Gangulee (1926), motile bacteria propel themselves in the water films that exist in soil, and in so doing may achieve an effective migration rate of 1 in per day. Additionally to active motility, cell proliferation and elongation, particularly of filamentous bacteria, leads to some increase in the bacterial colonization of soil. This mechanism of extension through soil becomes more effective in the presence of filamentous fungi possessing rapid growth rates. Such hyphae serve as roadways along which the bacteria follow, dining upon them *en route*. Finally, bacteria may be transported long distances above soil by airborne dust, by man-made machines, and by the migratory activity of large animals.

These several dispersal mechanisms have in the aggregate widely seeded bacteria throughout the soils of the world. Consequently, only rarely need soil inoculation be practised. Indeed, the population naturally present in a given habitat is almost invariably the one best adapted to exploit the habitat, and introducing one or more additional species of bacteria simply by inoculation is usually wasted effort. Either naturally occurring or man-induced changes in the habitat, however, are quickly followed by changes in the qualitative nature of the soil flora, irrespective of whether or not inoculation is practised.

The cosmopolitan nature of the bacterial flora in soil attests to widespread distribution of the organisms involved, but it is uninformative as to the efficiency of the several dispersal mechanisms. The present-day distribution

may have required thousands of years. Much more precise information is needed concerning the extent and rate at which bacteria can transport themselves or be transported within soil.

There is evidence that bacteria do not move freely into or through the soil water. Soil is well known for its capacity to entrap the bacteria which it receives in sewage effluents. In the course of nitrification experiments in a soil perfusion apparatus, Quastel and Scholefield (1951) observed that heavy populations of nitrifying bacteria became established on the soil particles while the perfusing liquid itself remained practically free of bacteria. The writer has observed that in a soil initially free of soybean rhizobia, uninoculated border or buffer rows intervening between inoculated (and nodulated) rows of soybeans remained uniformly free of nodulation throughout the growing season. This was surprising, inasmuch as at the time the soybeans were about 4 in high, the farm operator, faced with a heavy work schedule, turned in excessive heads of irrigation water and achieved a basin or flood irrigation, rather than a furrow irrigation along the rows. In the course of a single growing season, neither such flooding, nor any combination of dispersal mechanisms, sufficed to cause movement of rhizobia between nearly contiguous rhizospheres.

VII. MOISTURE REQUIREMENTS

The role of moisture in relation to microbial activity in soil has been studied extensively, possibly more so than has the influence of any other environmental factor. Notwithstanding such effort, there remain many gaps in knowledge concerning the soil water in terms of its interactions with other physical or biotic factors. Many of these gaps concern wet or extremely wet soils, in which the problem is really not one of moisture in itself, but of the severe restrictions imposed on aeration in wet soils.

Much of the earlier work on soil moisture in relation to bacterial activity is difficult to interpret. Moisture contents were expressed gravimetrically and without regard to the energy concept, in which soil moisture is expressed in terms of the physical force with which it is held in soil rather than in terms of actual percentage content. In some work, there was failure to insure a constant moisture content throughout an experimental period or to effect a uniform distribution of moisture throughout the experimental sample. In other work, data accumulated are informative only for the given experimental conditions. The rather widely quoted work of Bhaumik and Clark (1948), for example, is informative for soils maintained in a shallow layer of 12 mm and subjected to a rapidly moving air stream across the soil surface. What happens at the same moisture tensions in the field at a depth of 100 mm can hardly be extrapolated from such laboratory data.

The most satisfactory information on the moisture relationships of bacteria in soil is that which exists for the dry end of the moisture scale. Here the problem becomes simplified to definitions of the levels at which various bacteria can no longer hold water within their cells in amounts sufficient for their

metabolism. At moisture tensions of 3 atmospheres or higher, bacterial activity in soil becomes reduced, and very markedly so at the 15 atmosphere, or permanent wilting percentage, moisture content. Inasmuch as bacteria differ in the extent to which they are active in droughty soil, not all bacterial transformations are uniformly curtailed during drying out of soil. Ammonification, for example, can proceed under more stringent drought than can nitrification. The latter, although it occurs slowly in soil at the permanent wilting percentage, does not occur in soil of lower moisture content. Ammonification has been observed to occur in soil with moisture content at one-half the wilting percentage (Robinson, 1957).

Degradation of organic residues can occur at moisture contents below that of the permanent wilting percentage. Bartholomew and Norman (1947) observed that the threshold moisture content for decomposition of many plant residues is approximately that of 80% relative humidity. Possibly the observed decompositions were initiated by fungi rather than by bacteria. From the practical standpoint, it must be emphasized that low moisture contents are generally limiting to microbial activity. As the moisture content drops to or below the wilting percentage, decomposition processes slow almost to a standstill. At 81% relative humidity, the rate of decomposition of oat straw was measured as less than 1% of that at the optimum moisture content (Table V).

It is probable that at least some soils, even when in an air-dry condition, permit a very low level of respiratory activity. Negligible but nevertheless measurable production of CO_2 has been observed by the writer for a soil containing 4·8% organic matter and holding 3% moisture following air-drying in the laboratory. The rate of CO_2 production was of the order of 0·0001 mg CO_2/g of air-dry soil per day. In the same study, several other soils containing approximately 1% organic matter and holding approximately 1% moisture in the air-dry state showed no measurable production of CO_2 during 2 weeks of incubation.

TABLE V

Carbon dioxide evolution from oat straw under differing moisture conditions (Bartholomew and Norman, 1947)

% Moisture in oat straw	Moisture tension (atmospheres)*	Relative humidity	CO_2 evolved/g of straw, mg in 144 hr
250		saturation	152
150		saturation	139
60		saturation	79
36	40	97·1%	40
24	113	92·0%	16
19	206	85·9%	4
16	289	80·8%	1

* Values in this column calculated by the writer; not shown in the initial publication.

There occurs a marked reduction in the number of bacteria in soil as it undergoes drying. Certain bacterial species are commonly believed more resistant to drying than are others, and that consequently, sporulating and other drought-resistant bacteria constitute the bulk of the remaining viable population. This point of view is an over-simplification. All of the soil bacteria are quite resistant to drying. Seldom are individual species entirely eliminated from soils that are stored in an air-dry condition. When such soils are re-wetted, such varied phenomena as nitrification, ammonification, non-symbiotic nitrogen fixation and sulfur oxidation almost invariably proceed without any soil reinoculation whatsoever being required. Calder (1957) noted that the nitrate productivity of dry soil stored for 3 years was almost unimpaired. Sen and Sen (1956) encountered two soils in which *Rhizobium japonicum* survived for 19 years during storage of the soils in an air-dry condition. The writer has noted survival of *Azotobacter* in soil stored air-dry in the laboratory for 30 years.

These citations should not lead the reader to assume that specific bacteria are never entirely eliminated from soil as it undergoes drying. Workers engaged in nitrification studies and making use of stored dry soil at times find that some soils, or possibly only a single replicate of several small lots taken from a larger gross lot, do fail to show nitrification when properly moistened and incubated. Occasionally rhizobia may also fail to withstand soil drying. In some field soils cropped to annual legumes, especially if such soils are poorly buffered and subjected to severe drought during the non-growing season, there may be insufficient carryover of viable rhizobia to effect nodulation of the newly planted crop. Consequently, inoculation of the legume seed with rhizobia must be undertaken annually at time of planting.

When a soil is dried rapidly, it is not uncommon to note that such widely differing subgroups as spores of aerobic bacilli, nitrifying bacteria, denitrifying bacteria, and aerogenic bacteria respond uniformly with decrease in the total count. The decrease in the viable count is not linked to unilateral survival of sporulating bacilli.

For many soils, the decrease in bacterial numbers during drying is more apparent than real, and hinges on some physical entrapment or clumping effect, rather than on any true lethality. If, following drying, special mechanical or chemical dispersing treatments are applied to the dry soils prior to making plate counts thereon, the viable counts in the several soils are then higher, with magnitude of the increase differing in different soils, than counts obtained on aliquots of the same dry soils not given dispersing treatment prior to plating. Differences in physical entrapment effects among soils during drying can be expected to occur. Such differences may help to explain why different soils show widely varying decreases in total counts during drying.

VIII. AERATION REQUIREMENT

Wet soils are unfavorable for most bacteria simply because filling up of pore space with water diminishes soil aeration. The restrictive factor is lack

of oxygen and not the excess water in itself. At first glance it appears that soils with moisture content at or lower than field capacity could be expected to furnish oxygen to bacteria at a rate adequate to meet their biochemical oxygen demand. Seldom do field measurements of oxygen in the soil atmosphere reveal the presence of less than 10% oxygen; for most well-drained soils, the measurement usually is of the order of 20% (Russell, 1950). During wet periods and for heavy soils (Boynton and Compton, 1944; Vine *et al.*, 1942) or following heavy irrigation (Kemper and Ameniya, 1957), oxygen contents of 3% or below have been measured. These values represent oxygen contents measured in a large soil volume and may be an over-estimate of the oxygen content in some of the smaller and less accessible pores. The amount of oxygen present in a soil microsite at any given time is a function of the biochemical oxygen demand of soil organisms (and plant roots if present) and of the rate of oxygen movement into the soil atmosphere and into and through the water barriers in soil.

Movement of oxygen into the larger pores of the soil atmosphere occurs readily by gaseous diffusion. The oxygen pressure difference necessary to cause adequate movement through air-filled pores need equal only 1–4% oxygen. Bacteria in the soil, however, are surrounded by water films, and rate of oxygen diffusion through water is only about one ten-thousandth as fast as the rate through air. Consequently, water barriers to oxygen movement at times become limiting for bacterial respiration.

On a gross scale, such barriers can be established at the soil surface either by heavy rains or by irrigation practices. In the event that a water seal is thus established at the soil surface, the amount of oxygen in the soil atmosphere does not provide an inexhaustible reservoir for soil organisms. Its disappearance depends on the rate of oxygen use by soil organisms and plant roots and on the amount of oxygen initially entrapped. This amount is determined by the depth of the water table, the fraction of the soil volume occupied by air, and the initial oxygen content of the entrapped air. In many soils the entrapped oxygen is sufficient to supply the biochemical oxygen demand from several days to a week.

A much more difficult problem is that posed by the water barriers that occur in and around individual soil pores and bacteria and the extent to which these barriers can limit movement of oxygen to bacterial cells. One can be reasonably certain that these barriers are of little or no consequence at the moisture content of the permanent wilting percentage, inasmuch as at such content only soil pores having radii of 1 μ or less remain water-filled. With moisture content at field capacity, however, pores with radii as large as 3–4 μ become water-filled. Combinations of such pores, without intervening larger pores, may lead to conditions of inadequate aeration. Short of waterlogged conditions, however, it is difficult to postulate any idealized geometry on which to base calculations of rate of oxygen movement into and rates of consumption within soil micropores. Such calculations have been made for root segments surrounded by rhizosphere bacteria and soil-water barriers. Using experimentally measured rates of oxygen consumption and basing calculations on

the logarithmic relation between diffusion coefficient and film thickness, Clark and Kemper (1966) concluded that critical oxygen levels could occur within the internal root cells when the water or water mucigel barrier immediately surrounding the root reaches thicknesses of 0·1–0·5 mm.

In many soils, it is highly probable that microsites of anaerobiosis occur in greater profusion in the plow layer than in the subsoil. Well-drained soils with droughty surfaces normally can be expected to be rather fully aerated throughout the entire profile. With either sufficient precipitation or irrigation to effect a temporary water seal at the soil surface, the plow layer loses its oxygen much more rapidly than does the deeper profile, simply because the greater number of micro-organisms, and thus the greater biochemical oxygen demand, occurs in the topsoil. In such instances, the subsoil is better aerated than is the topsoil.

The observed distribution of anaerobic bacilli throughout the soil profile suggests that oxygen-deficient sites must occur in the plow layer of field soils. The data in Table I show that anaerobic bacteria were found most numerous, both relatively and absolutely, in the upper profile. In the A_1 horizon, they constituted 25% of the total count for aerobic bacteria, and in the next three and successively deeper horizons, 21, 21, and 10%, respectively. Whether the anaerobes proliferate during the times when temporary water-seals develop at the soil surface or whether they proliferate on a more or less continuing basis within microsites whose oxygen is consumed by aerobic bacteria is not currently known.

It is well known that nitrates are produced in the plow layer of field soils and accumulate therein unless taken out by plants or by leaching. The oxygen level required for nitrification is distinctly higher than that tolerated by obligate anaerobes. That both nitrifying and anaerobic bacteria develop in the plow layer suggests either that microsites of aerobiosis and anaerobiosis exist contemporaneously, or else that some sites fluctuate between aerobiosis and anaerobiosis under changing soil conditions.

Workers attempting to define the optimum soil atmosphere for either microbial transformations or microbial species have commonly placed major if not sole emphasis on the factor of oxygen content. The fluctuations that occur in the carbon dioxide content are also deserving of attention. Either unusually high or low carbon dioxide concentrations in the soil atmosphere at times are limiting for bacterial activity. Much of the work determining tolerances of soil organisms to high concentrations of carbon dioxide has been with the soil fungi rather than with the soil bacteria. Studies on threshold and optimal levels of CO_2 more frequently have been concerned with a few pathogenic bacteria rather than with the soil bacteria. Some information, however, has been developed concerning the CO_2 requirements of the nitrifying bacteria.

In the course of numerous studies by various workers on nitrification in soil, it has been recognized that incubation of soil samples with too shallow a layer of soil or with too small a quantity of soil in the incubation container, or with too vigorous aeration of the incubating soil, adversely affects the rate

of nitrification (Harmsen and van Schreven, 1955). Recent work indicates that the oxygen contents that exist under such differing conditions of incubation are not critical, but that build-up of soil carbon dioxide to a value above the normal atmospheric level is needed in order to secure an optimum rate of nitrification (Beard and Clark, 1962). Incubation techniques that favored rapid escape from soil of the respiratory carbon dioxide depressed rate of nitrification, and experimental removal of CO_2 from the soil atmosphere almost completely inhibited nitrification. These effects of CO_2 concentration could be observed at normal or near normal atmospheric oxygen levels. Oxygen itself became limiting only when its concentration by volume in the enclosed atmosphere fell to 10% or less. Nitrification rates in soils whose atmospheres contained concentrations of oxygen in the range of 10–20% by volume did not differ significantly. CO_2 content necessary for rapid nitrification fell within the range of 0·5–5·0% by volume.

Until more is known of the gaseous environment confronting the soil bacteria, one must be content with the generalization that soil moisture contents within the range of 50–80% of moisture-holding capacity appear compatible with good soil aeration. At least in soils of such wetness, there occurs a combination of moisture and aeration that favors the activities of most of the heterotrophic bacteria in soil.

IX. REACTION AND TEMPERATURE REQUIREMENTS

The reaction range commonly tolerated by soil bacteria is that between pH 4 and pH 10. The optimum within this range is slightly on the alkaline side of neutrality. Some bacteria are readily limited by acidity or alkalinity while others show wide tolerances to extremes in reaction. Such differences exist even within a single genus. *Azotobacter chroococcum*, for example, is widely distributed in neutral and alkaline soils but is not found in soils below about pH 6. *A. indicus*, in contrast, tolerates an acidity of pH 3. *Thiobacillus thiooxidans*, an autotroph capable of oxidizing elemental sulfur, tolerates an acidity of ph 0·6. It is reported to be the most acid tolerant of any living organism (Breed *et al.*, 1957).

As a group, the soil bacteria are less well adapted or less competitive for food supplies in distinctly acid soils than are the soil fungi. This is particularly evident in the microflora of forest and heath soils. In soils strongly acid in the surface profile, fungi are dominant, and even if the fungi are restricted experimentally by the addition of a fungicide, the pH alone suffices to restrict any vigorous colonization of the habitat by bacteria.

The optimum temperature range for soil bacteria is from about 25°–35° C. A great many grow quite well over the range of 10°–40° C. In the field, high soil temperatures are seldom if ever the primary factor limiting bacterial growth. Unusually high temperatures occur only at or near the surface in dry and barren soils. In such sites, lack of water is the primary factor limiting microbial growth. In moist tropical soils in which plants are growing, the soil temperature in the root zone is usually either optimal or suboptimal for

bacteria. Some arable soils, if row-cropped, clean-tilled, and not shaded by the growing crop, at times slightly surpass the optimum temperature for soil bacteria. Temperatures of this magnitude may be harmful to plant roots and therefore of economic importance. Their occurrence is usually avoided by corrective or alternative methods of land management.

On a limited scale, temperatures above the optimum for bacteria occur in soils or waters under the influence of thermal hot springs or volcanic activity. Stored hay and grain, if not sufficiently dry to inhibit microbial activity, may yield sufficient heat during spoilage to be inimical to many species of bacteria and even to cause spontaneous combustion in the stored material itself. Small islands of plant residues in soils, or layers of such residues that are plowed under, show measurable but inconsequential microbial thermogenesis (Clark *et al.*, 1962). Such temperature elevations are very transitory and do not exceed about 1° C.

In other tropical soils, temperatures below the optimum range for bacteria occur seasonally, and at time of occurrence are limiting to bacterial activity. The principal effect of seasonably low soil temperatures is simply to postpone microbial activity until a later date. Such postponement may have great economic importance in a cold, wet spring insofar as nutrient availability to a growing crop is concerned.

An extensive literature on the influence of temperature on diverse microbial transformations in soil has been reviewed by Richards *et al.* (1952), and their discussion will not be duplicated here.

X. BIOTIC LIMITATIONS ON BACTERIAL ACTIVITY

Emphasis in the preceding paragraphs has been on the extent to which the available food supply and physical factors in the environment determine bacterial activity in soil. Discussion of these factors severally or jointly fails to tell the full story, inasmuch as soil micro-organisms themselves exhibit pronounced effects upon one another. In some instances the biotic relationships may be beneficial to one or more organisms, but more often than not, mixed colonization of a substrate leads to restricted development of one or more of the species involved. Norman (1947) has characterized the soil population as one nutritionally fiercely competitive within itself. The extent to which the antibiotic and inhibiting substances are limiting to microbial activity in soil is discussed by Park in a later chapter.

The proposition that many of the microbial species present in a soil at any given time are in a resting or dormant condition has been put forward above. The competitive or ecological significance of such resting cells must be quite analogous to that of ungerminated but viable seeds in a plant community. Furthermore, among the active microbial species in soil, food specializations make possible the co-existence of a large number of ecological niches within any given habitat. In the rhizoplane, for example, with its wide assortment of materials exuded or sloughed from the root, it is probable that many

of the microbial species simultaneously present on the root surface are subsisting on different materials. The organisms occupy separate ecological niches and therefore are not in actual competition. Finally, any given substrate or microhabitat undergoes sequential changes with time, either because of fluctuations in the physical environment, or because of the activities of other micro-organisms. Such a substrate therefore presents a succession of ecological niches.

A simple illustration of one such succession can be seen in the nitrification cycle. Ammonia is oxidized to nitrite by the genus *Nitrosomonas*, and the nitrite to nitrate, by the genus *Nitrobacter*. Much more complex successions occur in the decomposition cycles of the organic residues that reach the soil. Many of these cycles have not yet been adequately studied. Recently, Kendrick and Burges (1962) have described the patterns of fungal specificity and succession that occur in decaying pine needles. Only by such studies, together with delimitation of the specific factors and mechanisms which enable one or another species to be found where it is found in soil, will the soil microbiologist be able to replace the redundancy in "this micro-organism is dominant in this microsite because it is the successful competitor" with a more meaningful conception of the role of competition in soil biology.

XI. PATTERNS IN MICROBIAL TRANSFORMATIONS

The many interactions of food supply with physical and biotic factors make each soil habitat, large or small, almost unique. At the same time, in the continual flux of microbial activity, some response patterns occur with such sufficient regularity that they are worthy of brief characterization. A few such response patterns are discussed in terms of CO_2 production, the parameter usually considered as the most informative concerning the course of organic matter decomposition in soil. When appropriate, some attention will be given to nitrification or other data. There is no implication that the few curves presented are all-inclusive for patterns of microbial activity, nor that any specific data, other than that given, will identify itself with any one curve. The rate curves shown, however, represent actual data taken from the literature, and are not constructed as idealized diagrams.

The principal food materials reaching the soil are plant and animal residues or excreta. These are almost continually added, and upon initial addition, contain soluble and easily available energy materials. The addition of such food materials to soil elicits therein the "microbial explosion" of which my former teacher Charles Thom so dearly loved to speak. Such explosions are characterized by a sudden onset of decomposition, the rise of the rate curve to a sharp peak within a day or so, and rapid subsidence of respiratory activity in the immediately following days, simply because the easily available food supply has been exhausted. Such decomposition curves are shown as the "A" curves in Figs 1 and 2.

Such data as are shown in Figs 1 and 2 represent total microbial respiratory

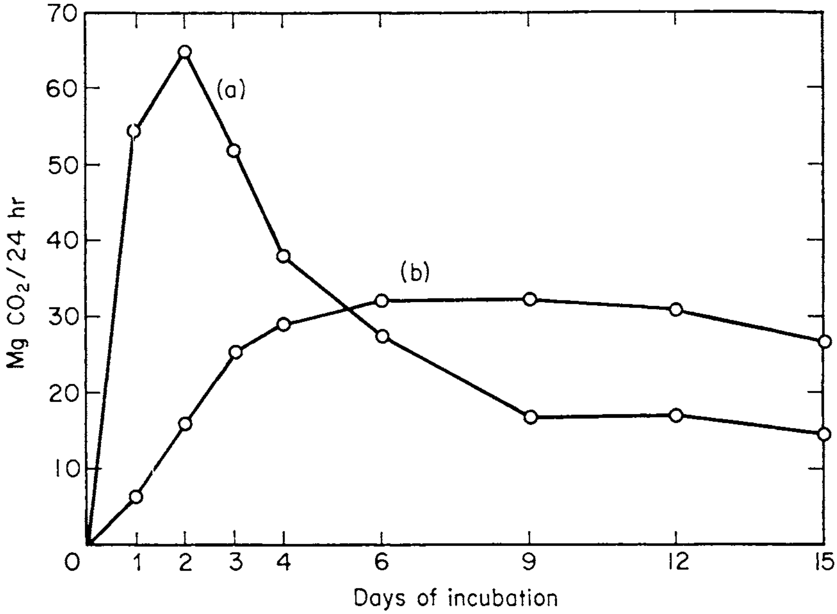

FIG. 1. Effect of two differing moisture tensions on the respiratory pattern in soil amended with 1% corn stover (Bhaumik and Clark, 1948).
(a) At 50 cm moisture tension; (b) at 0·0 cm moisture tension.

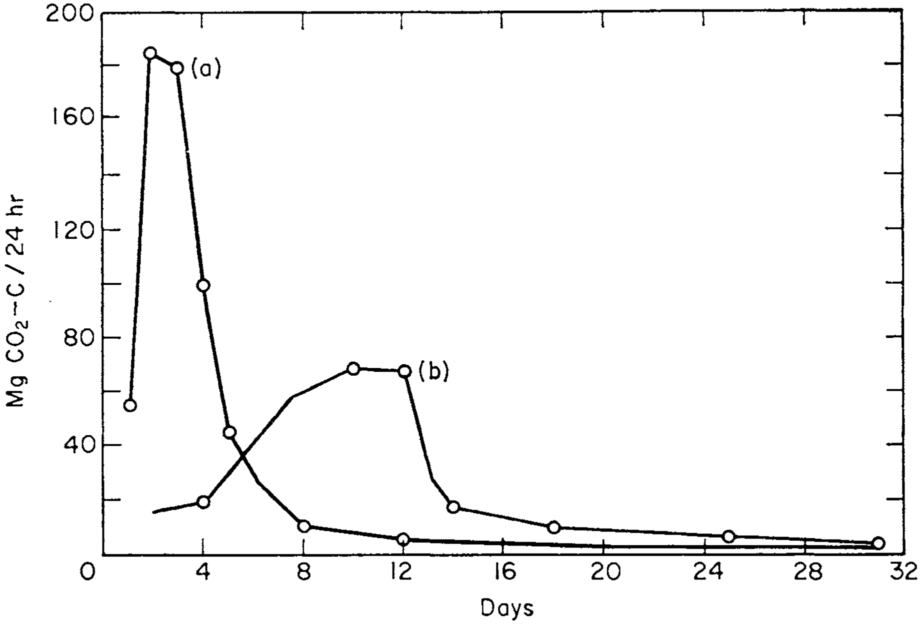

FIG. 2. Effect of additional nitrogen on the respiratory pattern in soil amended with 1% glucose (Stotzky and Norman, 1961).
(a) 1% glucose plus 0·15% nitrogen; (b) 1% glucose.

activity, and it is probable that much of the respiratory activity is by the soil fungi. However, it safely can be assumed that soil bacteria are also involved in the initial or explosive stage of decomposition of the added organic materials. Burges (1939) and Garrett (1956) have characterized the particular soil fungi involved in the early or rapid phase of decomposition as the "sugar fungi." Although their terminology might equally well be applied to the bacteria, the writer prefers not to speak of the "sugar bacteria." Nor for reasons to be discussed shortly does he concur in the designation of these rapidly responding bacteria as "zymogenous bacteria." Acceptance of the zymogenous and autochthonous classification of the soil bacteria as proposed by Winogradsky (1925) implies a rigidity of classification that does not exist. Bacteria that are zymogenous under one set of conditions may well be non-zymogenous under another set. It does, however, appear logical to speak of the explosive or peak activity that almost immediately follows the addition of fresh residues as the zymogenous response.

Under the influence of any one or more of a number of factors, the initial explosive activity or zymogenous response may be variously modified. Frequently, as some factor becomes limiting to decomposition, the ascending curve of respiratory activity breaks off to a horizontal plateau. With time, this limited activity accomplishes depletion of the food supply, and the respiratory curve drops off thereafter in much the same fashion as if the delaying factor had not intervened. Delayed or truncated patterns of zymogenous reponse are shown as the (b) curves in Figs 1 and 2. In Fig. 1, lack of adequate aeration due to excess moisture is the limiting factor; in Fig. 2, lack of essential mineral nutrient is responsible for the truncation.

Many organic residues that normally reach the soil contain, in addition to easily soluble and available components, others that are relatively resistant to decomposition. In plant residues, lignins and waxes commonly occur as such resistant material. They are responsible for the long-continued and relatively constant output of respiratory carbon dioxide following the transitory and explosive zymogenous response. The extent to which the water-soluble and non-water-soluble materials in corn stover contribute to the zymogenous and the residual patterns of response has been nicely demonstrated by Newman and Norman (1943). Their data are reproduced in Fig. 3. The water-extractables are responsible for the zymogenous peaks obtained on the first day, but after about 5 days their influence has been almost entirely dissipated, as shown by the coincidence of the respiratory curves for extracted and whole stover between the fifth and fourteenth days of incubation.

Some microbial transformations in soil show a lag pattern of response, or more descriptively, the growth curve pattern of response. This response is associated with the addition to a given soil of materials which are not commonly or frequently added thereto, or else if commonly added, only a few specialists in the soil population are capable of effecting their oxidation or hydrolysis. This pattern is characterized not only by a slow onset of microbial activity, corresponding to the lag phase of the well-known microbial growth curve for a population seeded into a fresh substrate, but also by the fact that

this lag or slow onset can be greatly foreshortened by pretreatment or baiting of the soil with the specific material.

A representative lag curve is obtained when a material such as a chlorinated organic pesticide is first added to a soil. Its decomposition occurs slowly. A second addition made shortly following the first addition is decomposed within a much shorter interval of time. Such a small first addition as one part per million of 2,4-D in soil suffices to increase the rate of decomposition of a second dose.

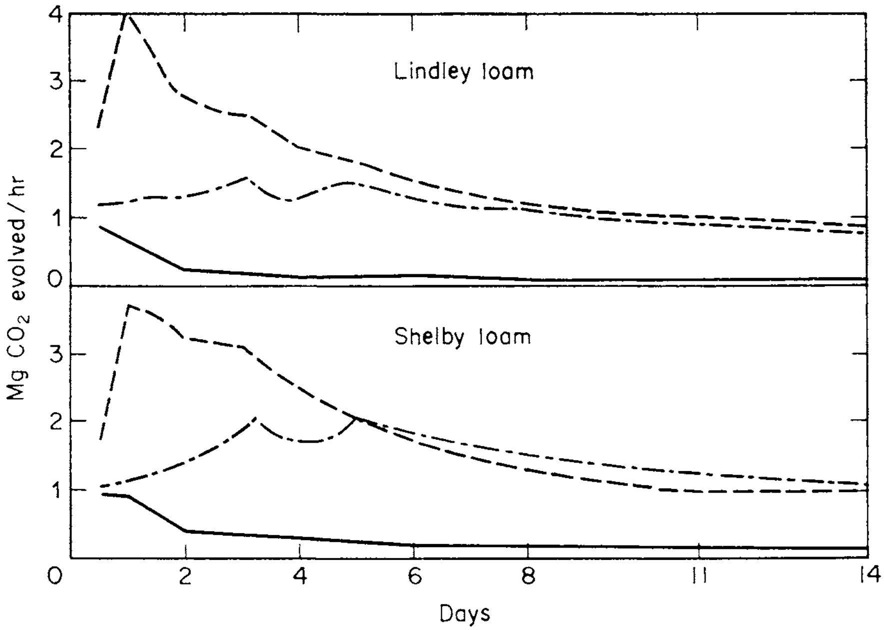

FIG. 3. Respiratory patterns in soils amended with extracted and whole corn stover (Newman and Norman, 1943).
Dashed line, soil and cornstalks; chain-dotted line, soil and water-extracted cornstalks; full line, soil alone.

A lag curve of response also is commonly observed when nitrifiable nitrogen is added to soil. A typical lag curve for nitrification in soil is shown in Fig. 4. The slow onset of nitrification is not due to any lack of nitrifiable nitrogen—indeed, the early part of the lag curve is entirely independent of the amount of nitrifiable nitrogen initially added.

The lag curve for nitrification in a laboratory soil perfusion apparatus is shown in Fig. 5. Additionally in this figure, there is shown the nitrification curve for ammonia added immediately following the completion of nitrification of a preceding addition.

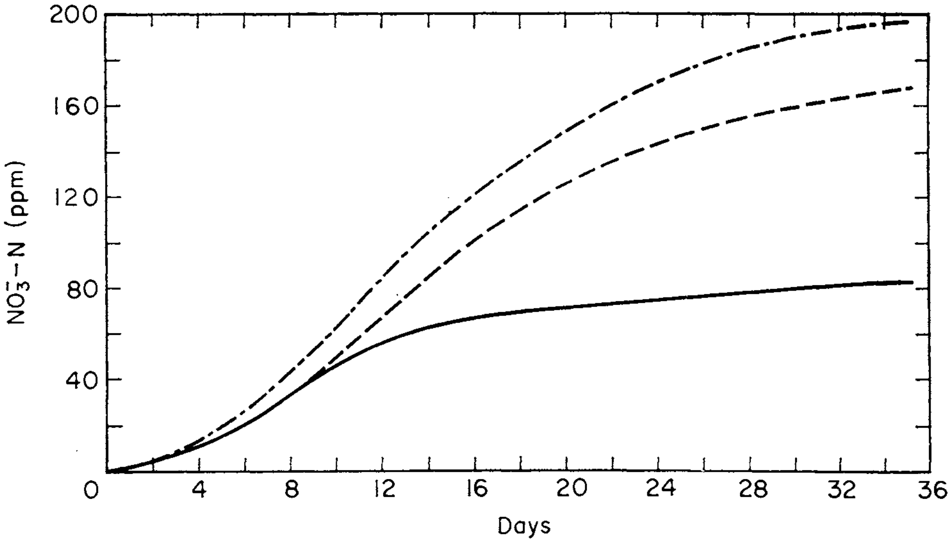

FIG. 4. Typical nitrification curves following additions of nitrifiable nitrogen to soil (Parker and Larson, 1962).
Chain dotted line, 150 ppm NH_4—N; dashed line, 100 ppm NH_4—N; full line, no nitrogen added.

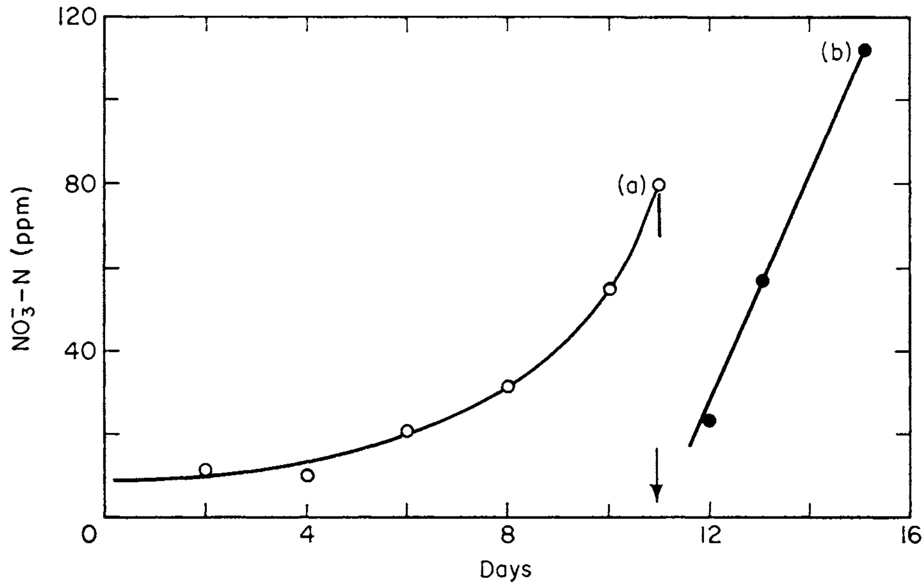

FIG. 5. Nitrification curves obtained during an initial and a second perfusion of soil with nitrifiable nitrogen (Quastel and Scholefield, 1951).
(a) Initial perfusion; (b) second perfusion of same soil.

The extent to which increasing the population level of the nitrifying bacteria shortens the lag portion of the nitrification curve is shown in Fig. 6.

In the lag response pattern, the responding bacteria must be considered normally to occur in soil at a low population level. The species involved are sufficiently slow growing that typically a week or more is required for them to reach any sizable population level even in the presence of ample energy material. Lees (1954) has commented on the slowness with which autotrophic bacteria such as the nitrifiers synthesize their cellular material.

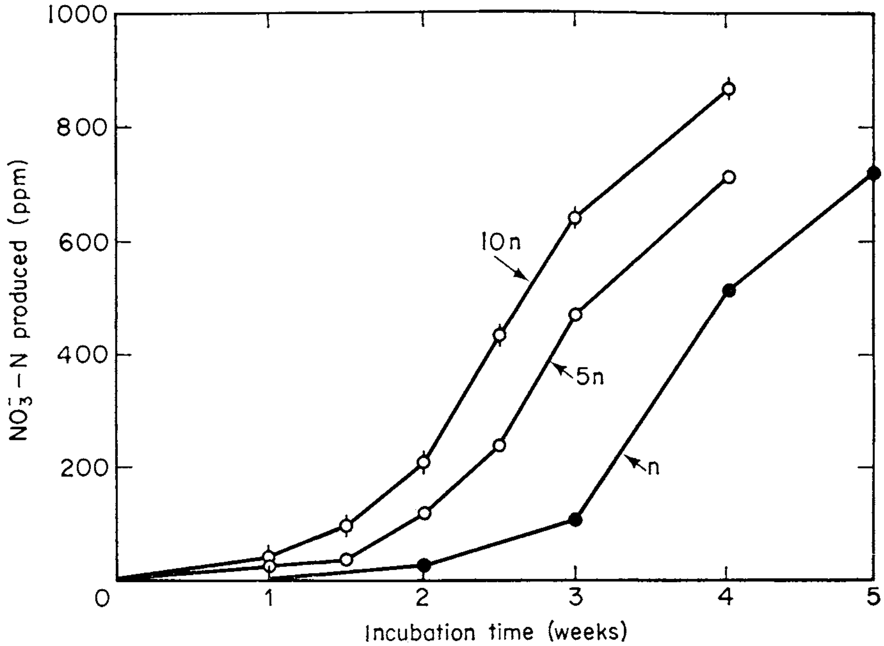

FIG. 6. Influence of initial population of nitrifying organisms on the nitrification pattern in soil (Sabey *et al.*, 1959).
n = 1 ml of inoculum of nitrifiers.

That slowly-growing bacteria participate in many transformations in the soil carries the implication that these forms are not faced with any appreciable competition for the substrate on which they subsist. Sugars, on the other hand, are susceptible to attack by a wide variety of heterotrophic organisms, and rapidity of response is mandatory for an organism if it is to compete successfully for a share of the added substrate. Another factor not to be overlooked is the frequency with which fresh residues reach the soil. As a consequence of this continued baiting, a microflora capable of zymogenous response is ever present in the average topsoil.

XII. THE PRIMING EFFECT IN DECOMPOSITION OF SOIL HUMUS

A characteristic of the soil humus is the slowness with which it decomposes. Half or more of the carbon in fresh residues is lost as carbon dioxide within 2–6 months following addition of the residues to soil. Thereafter, the rate of decomposition slackens. The ultimate product, the soil humus, decomposes at a rate of approximately 2% per annum. This rate is subject to some acceleration. For example, decomposition of the soil humus is intensified by thorough drying of the soil followed by re-wetting.

A concept developed relatively recently in soil microbiology is that addition to soil of fresh organic residues such as green manures accelerates the rate of decomposition of the native soil humus. The influence of the added residues has variously been termed as a fanning of the bacterial fires or as a priming effect. Studies of this phenomenon involve the use of isotopically tagged fresh residues in order that the total quantity of carbon or nitrogen mineralized during the course of an experimental incubation can be identified as arising from either the added residues or the native soil organic matter.

It is difficult to explain why the presence of freshly added residues should accelerate the decomposition of the native soil humus. Although residue addition produces a measurable thermogenesis, the limited elevation of the soil temperature is much too inconsequential to affect rate of decomposition. Nor should the flood of carbon dioxide released during decomposition of the added residues be expected to act as a microbial stimulant. A high concentration of this gas might well be equally detrimental or beneficial. Garrett (1956) has suggested that the observed intensification of decomposition of resistant materials in the presence of fresh materials hinges upon the necessity for supplying initial growth energy to the microbes involved in order to enable them to attack the resistant materials. Another possible explanation of the priming effect has been suggested by Park (1956). In his opinion, humus decomposition slows almost to a standstill because there accumulates in soil a miscellany of antibiotic or inhibitory substances. Once developed, these materials depress the rate of decomposition to a level lower than that which would otherwise occur. Addition of fresh residues, however, counteracts this biostasis, and thereby accelerates decomposition of the native humus.

It is the writer's opinion that the priming effect is largely illusory. This implies no challenge of the accuracy of the isotope measurements for evolved carbon dioxide or mineralized nitrogen, but it does imply doubt as to whether these measurements should be accepted at face value.

The total metabolic activity within any soil represents a complex of mineralizations and immobilizations in which the soil organisms draw upon available carbon and nitrogen, tagged or untagged, for their cell needs. In an unamended soil, all re-immobilization must occur at the expense of non-tagged carbon or nitrogen. In the presence of fresh residues, there is some channeling of the residues-added carbon and nitrogen to the immobilization pool. The full significance of these interchanges is as yet unknown. Investigations such

as that of Jansson (1960), in which an estimate was made of the active fraction of the soil humus by means of periodic additions of tagged glucose, should lead eventually to precise measurements of the interchanges between the soil and the added sources. Hauck and Bouldin (1961) have shown that dissimilar values for the nitrogen gas evolved from added nitrate in denitrification experiments are obtained according to whether or not one assumes dilution interchanges in soil of the original source material. Their work offers promise for a mathematical interpretation of the anomaly currently known as the priming effect.

XIII. SOME PROPOSED GROUPINGS OF THE SOIL BACTERIA

Any discussion of the bacterial flora of soil would be incomplete without some consideration of the approximate equilibrium among bacteria normally present in soil. What are the diverse types of bacteria in soil, and at about what frequency do some species of particular interest to the soil bacteriologist occur in soil?

Literally hundreds and possibly even thousands of bacterial species may be present in soil. It is not practical to discuss them individually. There are several ways in which the soil bacteria can be grouped together for convenience in discussion. Some groupings are too broad and too heterogenous to be informative—for example, the aerobic and anaerobic grouping with respect to oxygen tolerance, or the psychrophilic, mesophilic and thermophilic divisions based on temperatures at which growth occurs.

Within the past two decades, Canadian bacteriologists have made extensive use of a nutritional grouping of bacteria. The grouping initially proposed by Lochhead and Chase (1943) involved determination of growth responses of bacterial isolates on seven cultural media of increasing complexity. The types of cultural media required are shown in Table VI. Also shown therein are

TABLE VI

Growth requirements of soil bacteria (Lochhead and Thexton, 1947)

Nutritional group no.	Growth requirements	% Frequency in soil
1	Grow in basal mineral-salts medium plus energy energy	12·0
2	Require amino acids	6·8
3	Require growth factors	23·1
4	Require both amino acids and growth factors	16·2
5	Require yeast extract	16·2
6	Require soil extract	6·8
7	Require both yeast and soil extract	11·1

data by Lochhead and Thexton (1947) on the frequency of occurrence of soil bacteria in each nutritional category.

Later writers have criticized this classification and have proposed simplification. Taylor (1951) concluded that the nutritional groupings as proposed by Lochhead and associates yielded little information of value and did not allow bacterial groups to be distinguished with accuracy. Taylor proposed a modified scheme in which only five groupings were used. Later, Katznelson *et al.* (1956) made use of only the following three media: (a) basal medium consisting of inorganic salts and glucose; (b) this medium plus amino acids; and (c) the basal medium plus yeast and soil extracts. Gyllenberg (1957) made a different simplification; she used (a) basal medium; (b) basal medium plus amino acids; and (c) basal medium plus both amino acids and B vitamins.

At present there is no physiological grouping of the soil bacteria that will serve as a satisfactory substitute for their taxonomic grouping. Although it can be argued that the systematic identification of the wide assortment of bacteria in soil is not feasible for the individual worker, nevertheless the fact remains that in bacteriology, as in botany and zoology, the precision of the Linnaean system is needed in order that workers can effectively correlate their observations. Even though the individual worker cannot be expected to be proficient in making species identifications in more than a few genera, or indeed, to be interested in accomplishing identifications even in a single genus, nevertheless he should be able to recognize generic or some higher taxonomic category without too much difficulty.

Burges (1958) has noted that of approximately 1,600 species of bacteria and actinomycetes named in the sixth edition of *Bergey's Manual* (Breed *et al.*, 1948), approximately 250 species are listed as being typically soil-inhabiting forms. These were distributed among 50 genera. On the basis of these figures, slightly less than 16% of the named species are soil-inhabiting species. This percentage is probably an underestimate. Burges did not include in the soil flora isolates from muds and stagnant waters nor from decaying vegetation. Another qualifying factor is the extent to which taxonomic bacteriologists have been concerned with bacterial species pathogenic to man, animals, or plants, and with species encountered in the preparation and preservation of foods and in industrial fermentations. Intensive studies on bacteria in these categories have led to the naming of a great many species of non-soil origin. One can almost generalize that the "species splitters" have been in the ascendancy in the taxonomy of the plant and animal pathogens, and the "species lumpers," in soil bacteriology.

Using the seventh edition of *Bergey's Manual* (Breed, Murray and Smith, 1957), the writer has grouped the total number of bacterial genera listed as to whether or not they contain species of soil, or mud and pond water origin, or species occurring in association with plant debris in soil. Of a total of 190 genera, 97 genera, or 51%, contain species that broadly may be considered as soil bacteria. Surveys of this type have only limited value insofar as discussion on what actually occurs in the soil is concerned. If of 100 species listed

as of soil origin, 99 collectively account for only a fractional percentage point of the bacterial population in soil, obviously the full story is not told simply by summarizing habitat information.

XIV. ZYMOGENOUS AND AUTOCHTHONOUS FLORAS

Winogradsky's (1925) concept of a zymogenous and an autochthonous flora is an extremely useful one in characterizing the soil population as consisting of an indigenous component carrying on a low but rather constant level of metabolic activity and at the same time providing for the recognition of the zymogenous bursts of activity that follow the addition of fresh food or energy materials to soil. Attempts to carry his grouping beyond this point, however, appear to render more disservice than service to soil bacteriology. Particularly does this seem to be the case when attempts are made to sort out and assign individual genera and species to one grouping or the other.

The definition of the autochthonous or indigenous bacteria as those always present in soil but not fluctuating much in numbers, and carrying on activities which require no nutrients or sources of energy other than those normally present in the soil, is quite uninformative. All soil bacteria normally require only those nutrients or sources of energy normally present in the soil at one time or another, and all soil bacteria fluctuate in numbers when given the appropriate conditions. Characterization of the zymogenous bacteria as those actively fermenting forms which require for their activity ingredients that are quickly exhausted at first sounds plausible. It sounds distinctly less so when one finds that assignments to this group include not only the rapidly growing saccharolytic and proteolytic bacteria in a broad and sweeping gesture, but also along with them the vegetative but not the sporulating stages of *Bacillus*, members of *Pseudomonas*, the nitrogen-fixing bacteria as a group regardless of whether they are symbiotic or nonsymbiotic or aerobic or anaerobic, the cellulose-destroying and the sulfur-oxidizing bacteria, and finally, such difficultly cultured, fastidious and relatively scarce autotrophs as the ammonia- and nitrite-oxidizing bacteria (Conn, 1948; Alexander, 1961).

Could one isolate two soil bacteriologists in separate cubicles, and assign one to study the autochthonous and the other to study the zymogenous flora of the soil, it would be surprising if given sufficient time either investigator omitted studying any appreciable segment of the total bacterial flora of soil. Indeed, microbiologists who have endorsed the zymogenous and autochthonous groupings of Winogradsky often make contrasting assignments of components of the soil flora. Conn (1948), for example, considered the nitrifiers and the sulfur-oxidizers as zymogenous, whereas Burges (1958) regards the nitrifying bacteria, and the autotropic bacteria generally, as true autochthonous forms. Both Winogradsky (1925) and Conn (1948) considered the small coccoid bacteria of soil that are now assigned to the genus (*Arthrobacter* as true autochthonous bacteria. Rovira (1956), however, who considers the rhizosphere flora as zymogenous, has assigned (Sperber and Rovira, 1959) most of the Gram-negative bacteria in the rhizosphere to the genus

Arthrobacter. Katznelson and Sirois (1961) consider *Arthrobacter* at times to constitute an important fraction of the rhizosphere flora, and Jagnow (1961) assigned the great majority of his rhizosphere isolates to this same genus.

For the most part, it appears that those bacteria whose specific and favorable environments have been defined are commonly listed in the literature as the zymogenous flora. Significantly, this listing now includes practically all of the better known species of soil bacteria. These lesser known species whose ecological environments have been less adequately defined constitute the bulk of the autochthonous flora.

Given the proper conditions, any bacterial species in soil will show zymogenous response. Within the genus *Bacillus*, for example, such well-known species as *B. cereus* and *B. subtilis* commonly are termed zymogenous. If one adds 1% sucrose to soil, there occurs a profuse development of these two species. If, however, 1% peptone is added to soil, *B. sphaericus* and other round-spored bacteria become dominant. In the rhizospheres of plants, there is preferential stimulation of such species as *B. polymyxa*, *B. circulans*, and *B. brevis* (Clark and Smith, 1950). Without turning to other genera for further examples, it can be stated that specificity of response almost invariably occurs following development of special environmental situations in soil. Perhaps one of the most widely known relationships in this category is the host-bacterial symbiont specificity that occurs in the rhizospheres of leguminous plants.

Rather than to consider the soil bacteria as divisible into an autochthonous and a zymogenous flora, and then to attempt to assign individual species or genera to one grouping or the other, the writer prefers to think of all bacteria whose normal and principal habitat is soil as indigenous or native. This flora at any one time is composed of two segments. One of these can be termed a resting, and the other, a responding flora. As the soil environment undergoes change, first one group and then another finds conditions which enable it to undergo a burst of activity, that is, to exhibit the zymogenous response as emphasized by Winogradsky.

XV. THE PREDOMINANT BACTERIA IN SOIL

There is extensive evidence that the bacteria most abundant in soil are small coccoid rods of variable morphology (Conn, 1928; Jensen, 1933; Topping, 1937; Taylor, 1938; Gibson, 1939; Lochhead, 1940; Clark, 1940). These bacteria produce small colonies on such agar media as are commonly employed for total bacterial counts on soil. Their pleomorphism is extreme. Both their Gram-staining and their cellular morphology vary with age of subculture and with the substrate on which the bacteria are grown. Cells from very young cultures are usually Gram-negative rods, with or without rudimentary branching. Cells from older colonies on favorable substrates almost always appear as Gram-positive cocci. Taxonomically, the majority of the globiforme bacteria in soil are placed in the genus *Arthrobacter*, in the family *Corynebacteriaceae* of the *Eubacteriales*. Those species which decompose

cellulose are placed in the genus *Cellulomonas* within the same family (Breed *et al.*, 1957).

The soil corynebacteria usually constitute half or more of the total colonies encountered in the course of making plate count estimates of bacterial populations in soil. Taylor (1938) examined a wide variety of soils whose locations ranged from the rim of the Arctic Circle to southern Canada, and from the Atlantic to the Pacific coasts. Of 90 soils examined, globiforme bacteria were encountered in 89, and in these, constituted on the average about 65% of the total plate count populations determined. Numerous other workers dealing with arable soils of temperate regions have made essentially similar observations.

The extreme pleomorphism of the soil globiforme group makes extremely difficult the proper assignment to this group of the bacteria seen in direct microscopic examinations of soil. Burges (1958) has suggested division of the soil bacteria seen in direct microscopy into the following six groups:

1. small cocci about 0·5 μ in diameter;
2. short rods about 0·5 μ in diameter, 1 to 3 μ long;
3. short curved rods, the Vibrios;
4. long rods;
5. rods sometimes showing branching;
6. thin flexible rods with very thin walls, usually under 0·5 μ in diameter.

In this scheme, bacteria assigned to groups 1, 2, 4, and 5 could quite commonly be expected to be members of *Arthrobacter*, and occasionally, some of the asignments to groups 3 and 6. Similarly, in a morphological grouping made by Lochhead (1940) of the bacteria found in several soils in which plants were growing, the first five of the eight groups established (see Table VII) may well have been composed largely of the soil globiforme bacteria. These five groups constituted about 80% of the total number of bacteria examined.

XVI. OTHER BACTERIA ABUNDANT IN SOIL

Other bacteria abundantly present in soil are the sporulating bacilli and the actinomycetes. The latter commonly constitute at least 10% and at times as much as 70% of the total microbial count in soil. The actinomycetes are discussed by Kuster in a following chapter.

A cross-section of the census data available for soil bacteria shows the sporulating bacilli to constitute approximately 25% of the total number of bacteria culturally enumerated in soil. Mishustin (1956) has conducted population studies on a wide variety of soil types, ranging from tundra to desert. His data show that the bacterial spores constitute from 1 to 38% of the total bacterial count, with a mean percentage value of 24·2. Two of the tables that have been presented above show data on the occurrence of the genus *Bacillus* in soil. Table VII shows only approximately 10% of the total as sporeformers, but Lochhead's study was based on bacterial isolates from cropped soils, and

it is well known that the rhizosphere does not preferentially stimulate the sporulating bacteria as a group. The relative abundance of *Bacillus* in rhizosphere and non-rhizosphere soil is shown in Table IV.

TABLE VII

Frequency of occurrence of morphological types of bacteria predominantly present in soil (Lochhead, 1940)

Morphological classification	% of bacteria in each class Tobacco soil	Corn soil	Flax soil
Short rods, Gram-positive	23·3	23·1	7·7
Short rods, Gram-negative	13·3	26·9	15·4
Short rods, Gram-variable	11·7	5·8	7·7
Short rods, changing to cocci	13·3	7·7	1·9
Coccoid rods, Gram-positive	18·3	21·2	53·8
Cocci	1·7	0·0	7·7
Long, non-spore-forming rods	6·7	5·8	0
Spore-forming rods	10·0	9·6	5·8

Another group that is commonly present in soil is the mycobacteria. Censuswise their population density is less than that of the corynebacteria or the bacilli. It is difficult to place any precise estimate on their number, partly because of a paucity of published data, and partly because the more optimistic population estimates are by workers who have drawn wide boundary lines for this group of organisms.

XVII. WELL-KNOWN GENERA NOT ABUNDANTLY PRESENT IN SOIL

If one is to admit that the coryneforms account for as much as 65% of the bacterial flora exclusive of the actinomycetes in soil, and that the sporulating bacilli can account for another 25%, then collectively the *Pseudomonadacae*, *Nitrobacteriaceae*, *Rhizobiaceae*, *Azotobacteriaceae*, *Achromobacteriaceae*, and *Micrococcaceae* must account for no more than 10% of the total. On first thought, one would expect that by taking values for the occurrence of well-known genera within these families and summing up, the value so obtained would easily exceed 10%. Among the genera involved are *Agrobacterium*, *Azotobacter*, *Nitrosomonas*, *Nitrobacter*, *Rhizobium*, *Pseudomonas*, *Achromobacter*, and various others. Most of these have been studied intensively in relationship to specific transformations that they accomplish in soil or in their association with plants. With the notable exception of *Azotobacter*, the normal distribution in soil of many of these genera has not been studied intensively. Nevertheless, sufficient census data are available that some summing up can be accomplished.

Starkey (1931) enumerated *Agrobacterium radiobacter* in several soil samples collected from root-free soil. The average population of radiobacter encountered was 20,000/g of soil. The species accounted for no more than 0·1% of the total bacterial count determined on the same soil samples.

Azotobacter is usually present in soil at even lower population levels, and therefore constitutes an even more negligible fraction of the total count. Extensive data are available on the occurrence of *Azotobacter* in soil. Citation of a survey by Jensen (1950) on 264 Danish agricultural soils should suffice for purposes of this discussion. The following densities of *Azotobacter* were found:

Azotobacter per gram of soil	% of soils
0	46·2
100	26·9
100—1000	19·7
1000—10,000	6·1
10,000—100,000	1·1

The nitrifying bacteria also are commonly represented in soil by the thousands or ten thousands per gram. Only rarely are they to be found in excess of 100,000 per gram. *Nitrosomonas* and *Nitrobacter* together therefore can be expected to account for less than 1% of the soil population.

Holding (1960) has recently studied the abundance of Gram-negative bacteria in soil. He determined their frequency in the total bacterial population and, additionally, the frequency of occurrence of many individual genera. Some of his data are summarized in Table VIII.

TABLE VIII

Frequency of occurrence of Gram-negative bacteria in soil (Holding, 1960)

	% total microbial no. in		
	Plant-free soil	Rhizosphere soil	Soil with glucose added
Pseudomonas (all types)	5·53	13·6	12·4
Xanthomonas	0·28	1·2	
Chromobacterium	0·28	0·2	
Flavobacterium			1·0
Achromobacter	0·49	0·8	4·6
Cytophaga	0·42		
Agrobacterium		1·4	
Alcaligenes		1·0	2·0
All Gram-negative bacteria as a group	7·0	20·0	20·0

Holding's study reveals *Pseudomonas* as the only Gram-negative genus present in either rhizosphere or plant-free soil in excess of 5% of the total count. Clark (1940) has noted fluorescent bacteria, or *Pseudomonas* and *Xanthomonas* combined, to constitute from about 1 to 30% of the rhizosphere population of various plants.

The *Micrococcaceae* are rarely encountered in soil. Conn (1948) found the true cocci so rarely present that he doubted the few actually observed were of soil origin. Time and again, however, cultures were isolated from soil which seemed to be micrococci when first examined, but which on continued study were found to be the coccoid stages of *Arthrobacter*.

Recently, Stolp and Petzold (1962) have described some hitherto unrecognized obligately parasitic bacteria that possess lytic activity for bacteria of the genera *Pseudomonas* and *Xanthomonas*. That such parasitic or related types of bacteria may be encountered in the course of soil microscopy is suggested by recent observations of M. Fieldes.* He has observed spherical bodies or cells, about 0·25 μ in diameter and each bearing a single, heavy flagellum, in the course of examining electron-micrographs of carbon replicas of the surfaces of soil particles.

When considering the types of bacteria in any given soil, perhaps the safest course is always to expect the unexpected. Surveys that show the globiforme bacteria, the sporulating bacilli, or the fluorescent Gram-negative rods as the abundantly occurring bacterial flora in soil are summarizing generalities. Any or all of these types may be found only with difficulty in certain soils in which some combination of environmental conditions and type of energy material available may greatly favor relatively unknown or bizarre species. In such an event, bacteria commonly detected in soil only by special searching and enrichment procedure may become so numerous as to account for a major fraction of the bacterial biomass in soil.

* Personal communication, 1963.

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